The Development of Conspecific Interactions in Juvenile *Aplysia dactylomela* Rang, 1828: An Observational Study

BY

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(3 Text figures)

INTRODUCTION

THE PURPOSE OF THIS STUDY was to describe developmental changes in the interactions of "juvenile" *Aplysia dactylomela* Rang, 1828. It became possible with the discovery of an apparently "juvenile" population in the Plajita Rosada collection on the southwest coast of Puerto Rico during April and May 1975 (see Figure 1). This was an unusual research opportunity in that the capability of raising sea hares through metamorphosis has been restricted to only a few laboratories in which behavioral development was, to date, not investigated. The methods and procedures used were peculiar to an opportunity such as this, in that they depended on the continued success of finding "juveniles."

Aplysia californica Cooper, 1863 has been raised through metamorphosis and some of the morphological and behavioral changes related to metamorphosis have been described (KRIEGSTEIN, 1976; KRIEGSTEIN *et al.*, 1974). Further progress in the establishment of laboratory cultures of *Aplysia* has been reported by HADFIELD, (1975) and STRENTH & BLANKENSHIP (1976). Recently studies of morphological development have become available for some related opisthobranchs (THOMPSON, 1958, 1962, 1967; TARDY, 1970; BONAR & HADFIELD, 1974), but there are no published studies of behavioral development in the opisthobranchs.

The first occurrence of copulation in *Aplysia* seems to be related to size. Although no data were provided, in a brief report NEWBY (1972) stated that copulation was never observed in animals less than 40g body weight. SMITH & CAREFOOT (1967) collected small sea hares (1g-8g) and kept these animals together as a group. Copulation was not observed until the 16th day in the laboratory. The body weights at the time of copulation were not reported; however, copulation was observed at about the time the gonads became mature. Thus, it appears that the reproductive behavior develops some time after metamorphosis.

Morphological studies of the bag cells support the notion that the reproductive system as a whole may develop after metamorphosis. The bag cells are a neurohormonal group of the abdominal ganglion which contribute to egg-laying (ARCH, 1976). The cells are few in number and may be non-secretory in small animals (2g). In sea hares larger than 50g, however, secretory granules are present and the number of cells in the cluster increases markedly (FRAZIER, *et al.*, 1967). Thus, some neuroendocrine aspects of egg-laying become functional around the stage when sea hares begin to copulate.

Although the data are scanty, it appears from the above studies that copulation and size might be related. In this study the same sea hares were observed in regular repeated pairings. Systematic observations of their behavior and weight measurements were made.

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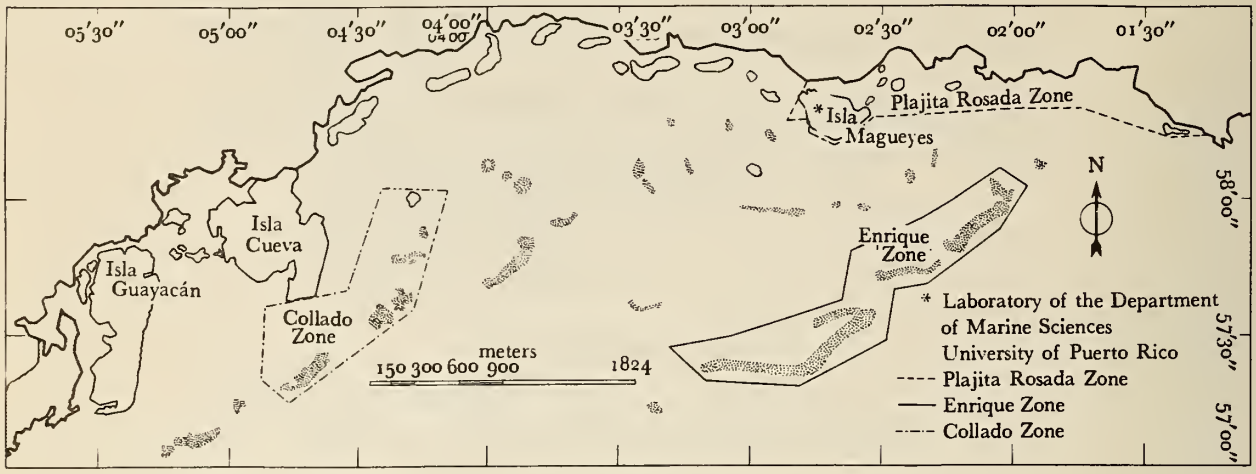

Figure 1

METHODS AND PROCEDURES

A. Subjects and Maintenance

A sample of 56 sea hares was collected off the southwest coast of Puerto Rico at Plajita Rosada. This is a rocky intertidal beach marked by the presence of *Laurencia papilosa* and sargassum (for details of collection see LEDERHENDLER, 1977).

The sea hares were maintained in an indoor laboratory in 1 *l*-capacity aquaria with running sea water and constant daylight illumination. Food was always available and consisted of *Acanthophora spicifera* which was thoroughly rinsed before being placed in the tanks. Wet weights of the animals were measured every other day on a Mettler balance.

To carry out observations of the interindividual behavior, the members of a pair were matched for size. As a result, the smallest animals were paired at the time of collection, to permit a maximum amount of observation during a long period of growth and development.

Five pairs were chosen for observation. These were all from the April and May collections (Figure 2). A failure in the sea water supply cut the period of observation short, resulting in making only 15 to 22 days available in which a total of 22 paired observations were made.

B. Apparatus and Procedure

Observations occurred between 5:00 p. m. and 7:30 p. m. under a 60-watt fluorescent bulb. The animals were paired by their size. Two pairs were observed for 30

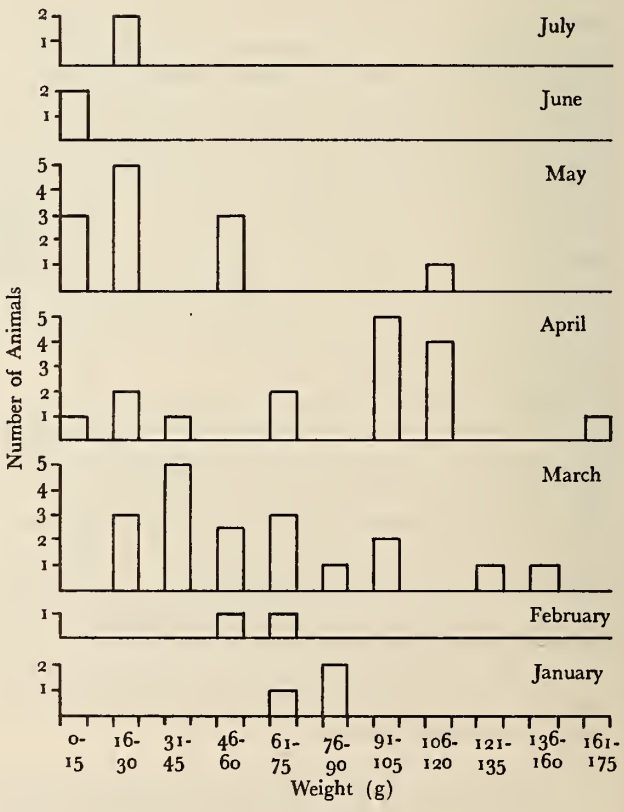

Figure 2

Frequency Distribution of Initial Weights of *Aplysia dactylomela* Collected at Plajita Rosada, Puerto Rico (N = 56)

minutes every 6 days; 2 pairs were observed every other day. The 5th pair was observed as individuals on experimental day 1 and then every 6 days (Table 1). A circular bowl 30cm in diameter and 10.2cm high was filled with fresh sea water to a depth of 8cm. The animals were taken from their home tanks and weighed. The observation began immediately upon the introduction of the second animal.

Table 1

Characteristics of Five Pairs of Pre-Copulatory *Aplysia dactylomela* Observed Repeatedly in Different Frequencies

	Initial weight (g)	Number of pairings	Interval between pairings
Pair 1			
Partner A	14	4	6 days
Partner B	16		
Pair 2			
Partner A	25	3	6 days
Partner B	26		
Pair 3			
Partner A	13	2	6 days[2]
Partner B	16		
Pair 4			
Partner A	6	7	2 days
Partner B	11		
Pair 5			
Partner A	21	6	2 days
Partner B	24		

[2]Unpaired on first experimental day.

The frequency and duration of contact and copulation as well as more detailed aspects of the interactions were recorded. Contact was defined as any part of an individual touching any other part of another individual. A sea hare initiated contact if it approached the other and touched it with any part of the body. Approach was defined as any locomotion which decreased the interindividual distance while oriented toward the second individual. Reciprocal contact occurred when both animals initiated the contact. Copulation was said to occur upon the intromission of one animal's penis into the common genital opening of the partner. Under the observational conditions described above, this could be determined exactly. If a pair was still in contact or copulating by the end of the observation, spotchecks every 5 minutes continued until the animals separated.

Table 2

Patterns of Contact and Copulation of "Juvenile" *Aplysia dactylomela* Paired Every Two Days

	Pairing						
	1	2	3	4	5	6	7
Pair 4							
Number of Contacts Initiated By Partner A	0	0	0	3	1	4	0
Number of Contacts Initiated By Partner B	1	3	2	6	2	2	3
Number of Contacts Initiated Reciprocally	0	1	1	0	2	2	2
Pair 5							
Number of Contacts Initiated By Partner A	2	0	0	2	0	0	—
Number of Contacts Initiated By Partner B	0	0	0	0	0	0	—
Number of Contacts Initiated Reciprocally	1	0	1	1	1	2	—
Sperm Recipient	—	—	B	B	B	A	—

C. Sample Characteristics

Figure 2 shows the distribution of weights for the population from which the experimental subjects were selected. The median weight of the entire group was 56g. The experimental sea hares were collected in April and May when most of the population (15 out of 28) were below 60g. The median weight of the experimental animals was 16g.

Only 1 pair of the 56 animals from Plajita Rosada was found copulating and no other animals were found in contact even though most were collected near each other, within approximately 75m of coastline.

RESULTS

Figure 3a - 3e shows the growth curves and duration of contact and copulation with repeated pairings for each pair in the study. As these data indicate, copulation occurred in only 1 pair (5). There appears to be a tendency for longer contact interaction with increasing size where-

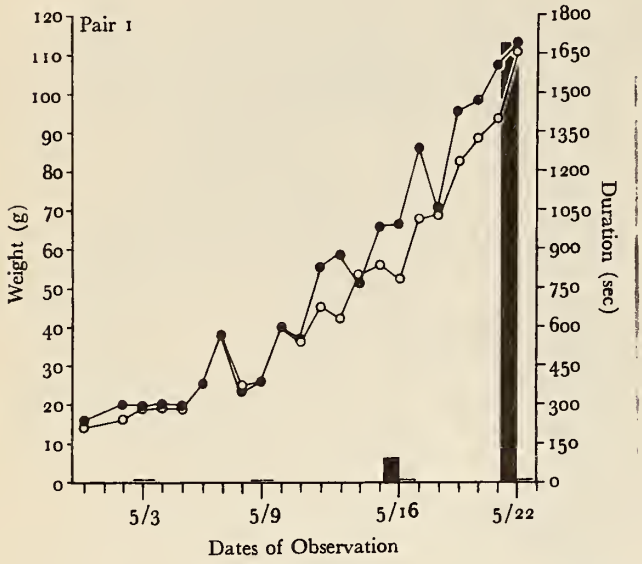

Figure 3a

Total duration (sec) of contact (solid bar) and weight changes of immature *Aplysia dactylomela* found at Plajita Rosada, Puerto Rico.

Copulation did not occur

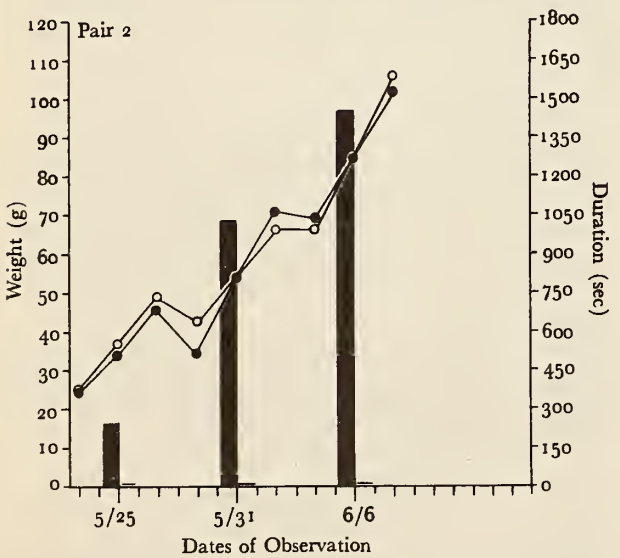

Figure 3b

Total duration (sec) of contact (solid bar) and weight changes of *Aplysia dactylomela* found at Plajita Rosada, Puerto Rico.

Copulation did not occur

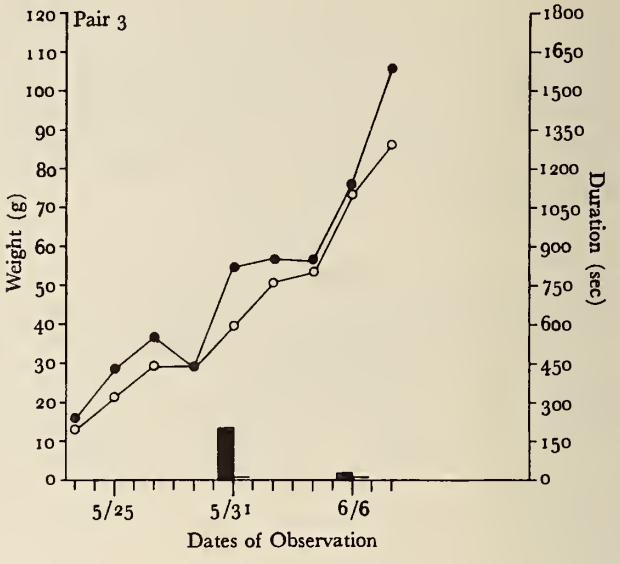

Figure 3c

Total duration (sec) of contact (solid bar) and weight changes of *Aplysia dactylomela* found at Plajita Rosada, Puerto Rico.

Copulation did not occur

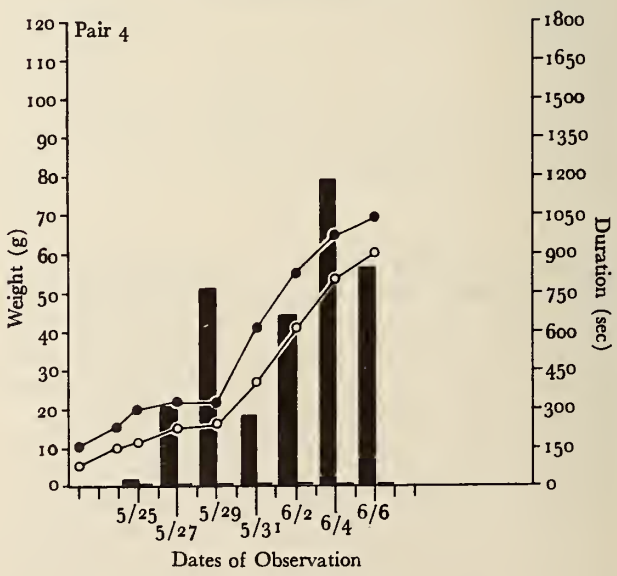

Figure 3d

Total duration (sec) of contact (solid bar) and weight changes of *Aplysia dactylomela* found at Plajita Rosada, Puerto Rico.

Copulation did not occur

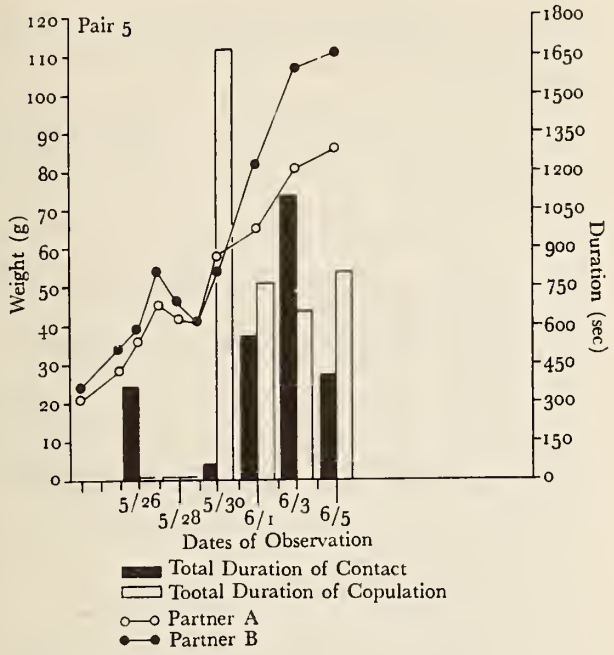

Figure 3e

Total duration (sec) of contact (solid bar), copulation (open bar) and weight changes of *Aplysia dactylomela* found at Plajita Rosada, Puerto Rico

ever there is a consistent tendency to contact at all.

Pairs 4 (Figure 3d) and 5 (Figure 3e) each had 7 and 6 observations respectively. This permitted somewhat more detailed statements about the interactions. As Table 2 shows, pairs 4 and 5 were different in their patterns of contact. In pair 4, a single contact, initiated by B, occurred early during the initial pairing and lasted for 35 seconds. The animals weighed 11 g and 20 g respectively during this observation. During the 1st 3 pairings, 2 contacts were initiated mutually; 6 other contacts were initiated only by Partner B (1 in the 1st, 3 in the 2nd, and 2 in the 3rd pairing). In the 4th observation B initiated 6 out of 9 contacts. In the 5th, B initiated 2 contacts, A initiated 1, and 2 were mutually initiated. In the 6th pairing, out of 8 separate contacts, B initiated only 2, 4 were initiated by A, and 2 were mutual. But in the 7th pairing B initiated 3 out 5 contacts where 2 were mutually initiated. Thus, out of 27 separate contacts which were not initiated by both animals (total of 8), B initiated 19 and A initiated 8.

In pair 5, contact occurred during the 1st observation when the pair weighed 28 g and 34 g respectively. There was no contact during the 2nd pairing. Considering all 6 observations, 6 contacts were initiated reciprocally and 4 were not. These 4 were all initiated by partner A, who was also the sperm donor in 3 of the 4 observation periods where copulation occurred. Partner B became the sperm donor only after A had assumed that role 3 times. The animals contacted shortly after the 3rd pairing and began to copulate after 90 seconds of the observation period had elapsed. At this time, they weighed 53 g and 58 g respectively. Copulation occurred in each of the next 3 pairings. The 4 copulations lasted for 43, 12, 21 and 49 minutes respectively. It is noteworthy that when partner B assumed the sperm donor role the duration increased to a level equivalent to the first copulation when A was the sperm donor.

DISCUSSION

The *Aplysia* observed in this study were assumed to be pre-copulatory because of the following: 1. Of 56 animals collected from the same location, only one pair was found copulating in the field; these 2 individuals weighed 47 g and 55 g respectively. In addition, none of the animals found in the field was in contact, although many were near each other. 2. Most of the sea hares did not copulate with repeated pairings in the laboratory. 3. None of the animals laid eggs while they were in the laboratory. Together with their small size when collected, these observations suggest that they were not reproductively mature.

Although only 1 of the 5 pairs copulated, it shared some characteristics with those that did not copulate, such as change in weight, duration of contacts, etc. However, it is useful to examine this pair in detail. For example, Frazier *et al.* (1967) found that the bag cells of *A. californica* begin to contain neurosecretory granules when animals reached 50 g in weight. Copulation occurred after each animal of pair 5 passed the 50 g mark in body weight, indicating that this may be a necessary but not sufficient condition for copulation to occur, as all pairs reached that weight before observations ended.

In the one pair which copulated, the animal which was the sperm recipient was heavier at the start (B). Following the first copulation there was a spurt in its growth rate in comparison with its partner (A's), and their congruent pre-copulatory pattern of weight change. If the spurt in weight proves reliably associated with copulation, the onset of egg-laying (which may depend on bag-cell hormones) could be related to copulation and the contacts which precede it.

Ledehendler *et al.* (1976) found that in reproductively mature *Aplysia dactylomela* animals which behave more consistently in the sperm recipient role copulate with more partners than the consistent sperm donors. They suggest that this may be based on a feedback system related to amount of sperm received. In the observations recorded here, role reversal occurred after 3 copulations in which the same roles were assumed. This is consistent with the function of such a feedback system. These observations are considered preliminary but indicate directions for further research on the possible interdependence of social stimulation and physiology in the development of reproductive processes in this species.

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